BioOcean® Human uPAR ELISA Kit

Catalog Number EK1171
Size: 96 Test

For the quantitative determination of human Urokinase-type Plasminogen Activator Receptor (uPAR) concentrations in cell culture supernates, serum and plasma.

This package insert must be read entirely before using this product. For proper performance, follow the protocol provided with each individual kit.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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QUICK PROCEDURE FLOWCHART

1. **Prepare all reagents and standards as directed.**

2. **Add 100 µl 2-fold diluted *Standard* in duplicate.**

3. **Add 90 µl *Assay Buffer (1×)* and 10 µl sample to the sample well within 15 minutes.**

4. **Add 50 µl diluted *Detect Antibody* to each well.**

5. **Incubate for 2 hours at RT.**

6. **Aspirate and wash 6 times.**

7. **Add 100 µl diluted *Streptavidin-HRP* to each well.**

8. **Incubate for 45 minutes at RT.**

9. **Aspirate and wash 6 times.**

10. **Add 100 µl *Substrate Solution* to each well.**

Incubate for 10 - 30 minutes at RT. Protect from light.

11. **Add 100 µl *Stop Solution* to each well.**

12. **Read at 450 nm within 30 minutes. Correction 570 or 630 nm.**

DESCRIPTION

Urokinase-type Plasminogen Activator Receptor (uPAR) is a cell surface receptor that binds urokinase-type plasminogen activator (uPA) with high affinity, thereby facilitating the pericellular activation of plasminogen. It is multidomain glycoprotein tethered to the cell membrane with a glycosylphosphotidylinositol (GPI) anchor. uPAR was originally identified as a saturable binding site for urokinase on the cell surface.

uPAR binds urokinase and thus restricts plasminogen activation to the immediate vicinity of the cell membrane. Besides the primary ligand urokinase, uPAR interacts with several other proteins, among others: vitronectin, the uPAR associated protein and the integrin family of membrane proteins.

uPAR is a part of the plasminogen activation system, which in the healthy body is involved in tissue reorganization events such as mammary gland involution and wound healing. uPAR has been involved in various other processes related to cancer, such as cell migration, cell cycle regulation, and cell adhesion.

PRINCIPLE OF THE ASSAY

BioOcean Human uPAR ELISA Kit is based on the quantitative sandwich enzyme-linked immunosorbent assay technique to measure concentration of human uPAR in the samples. A monoclonal antibody specific for human uPAR has been immobilized onto microwells. Standard or samples are pipetted into the wells, followed by the addition of biotin-linked monoclonal antibody specific for uPAR, and uPAR present is bound by the immobilized antibody and detect antibody following the first incubation. After removal of any unbound substances, streptavidin-HRP is added for a second incubation. After washing, substrate solution reacts with HRP and color develops in proportion to the amount of uPAR bound by the immobilized antibody. The color development is stopped by addition of acid and the optical density value is measured by microplate reader.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use expired kit or reagents.
- Do not use reagents from other lots or manufacturers. Do not prepare component by yourself.
- If concentration of assayed factor in samples is higher than the highest standard, dilute the serum/plasma samples with *Assay Buffer*, dilute the cell culture supernate samples with *cell culture medium*. Reanalyze these and multiply results by the appropriate dilution factor.
- Any variation in testing personnel, sample preparation, standard dilution, pipetting technique, washing techniques, incubation time, temperature, kit age and equipment can cause variation in results.
- Bacterial or fungal contamination in either samples or reagents, or cross-contamination between reagents may cause erroneous results.

MATERIALS PROVIDED

Unopened kit should be stored at 2 - 8℃.

- **uPAR Microplate** (1 plate): 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human uPAR.
- **uPAR Standard** (2 vials): Recombinant human uPAR in a buffered protein base with preservatives; lyophilized.
- **uPAR Detect Antibody** (1 vial, 80 µl): Biotin-conjugate anti-human uPAR monoclonal antibody; 100× liquid.
- **Standard Diluent** (1 bottle, 5 ml): In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent vial. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.
- **Streptavidin-HRP** (1 vial, 150 µl): 100× liquid.
- **Assay Buffer (10×)** (1 bottle, 5 ml): PBS with 0.5 % Tween-20 and 5 % BSA.
- **Substrate** (1 bottle, 15 ml): TMB (tetramethyl-benzidine).
- **Stop Solution** (1 bottle, 15 ml): 0.18 M sulfuric acid.
- **Washing Buffer (20×)** (1 bottle, 50 ml): PBS with 1 % Tween-20.
- **Plate Covers** (5 strips).

STORAGE

Store kit reagents between 2 and 8℃. Immediately after use remaining reagents should be returned to cold storage (2 to 8℃). Expiry of the kit and reagents is stated on labels.
Expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Unopened kit		Store at 2 - 8℃ (See expiration date on the label).
Opened/ Reconstituted Reagents	1× Washing Buffer 1× Assay Buffer Stop Solution Standard Diluent Substrate TMB Detect Antibody Streptavidin-HRP	Up to 1 month at 2 - 8℃.
	Reconstituted Standard	Up to 1 month at ≤ -20℃ in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
	Microplate Wells	Up to 1 month at 2 - 8℃. Return unused strips to the foil pouch containing the desiccant pack, reseal along entire edge to maintain plate integrity.

Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- **Microplate reader** capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- **Pipettes and pipette tips**.
- 50 µl to 300 µl adjustable **multichannel micropipette** with disposable tips.
- Multichannel micropipette **reservoir**.
- **Beakers, flasks, cylinders** necessary for preparation of reagents.
- **Deionized or distilled water**.
- **Polypropylene** test tubes for dilution.

PRECAUTION

- Intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Treat all chemicals with caution because they can be potentially hazardous.
- It is recommended that this product is handled only by persons who have been trained in laboratory techniques and in accordance with the principles of good laboratory practice. Wear personal protection equipment such as laboratory coat, safety glasses and gloves.
- Avoid direct contact with skin or eyes. Wash immediately with water in the case of contact with skin or eyes. Avoid contact of skin or mucous membranes with kit reagents or specimens. See material safety data sheet(s) for specific advice.
- Pure water or deionized water must be used for reagent preparation.
- The Stop Solution provided with this kit is an acid solution. Wear personal protection equipment with caution.
- Do not expose kit reagents to strong light during storage and incubation.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- Use disposable pipette tips and/or pipettes to avoid microbial or cross-contamination of reagents or specimens which may invalidate the test.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the HRP and antibody conjugate.
- Substrate solution must be warmed to room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5℃.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

TECHNICAL HINTS

- All reagents including microplate, samples, standards and working solution should be warmed to room temperature before use.
- To obtain accurate results, using adhesive film to seal the plate during incubation is suggested.
- It is recommended that all samples and standards be assayed in duplicate.
- Avoid foaming when mixing or reconstituting solutions containing protein.
- To avoid cross-contamination, use separate reservoirs for each reagent and change pipette tips between each standard, sample and reagent.
- When using an automated plate washer, adding a 30 seconds soak period before washing step and/or rotating the plate between wash steps may improve assay precision.
- When pipetting reagents, maintain a consistent order of addition from well-to-well.
- Keep Substrate solution protected from direct strong light. Substrate Solution should turn to gradations of blue after a proper color development.
- Read absorbance within 30 minutes after adding stop solution.
- Take care not to scratch the inner surface of the microwells.
- High background maybe occur when using some cell culture medium as standard diluent.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

Serum – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000 g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1000 g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay. Dilution with Assay Buffer maybe needed.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.
If samples are to be run within 24 hours, they may be stored at 2 to 8℃. For longer storage, aliquot samples and store frozen at -20℃ to avoid loss of bioactive human uPAR. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1000 ml graduated cylinder.
Bring to final volume of 1000 ml with pure or deionized water.
Mix gently to avoid foaming.
Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder.
Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.
Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube as needed.
The diluted Detect Antibody should be used within 30 minutes after dilution.

Streptavidin-HRP

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed.
The diluted Streptavidin-HRP should be used within 30 minutes after dilution.

Sample Dilution

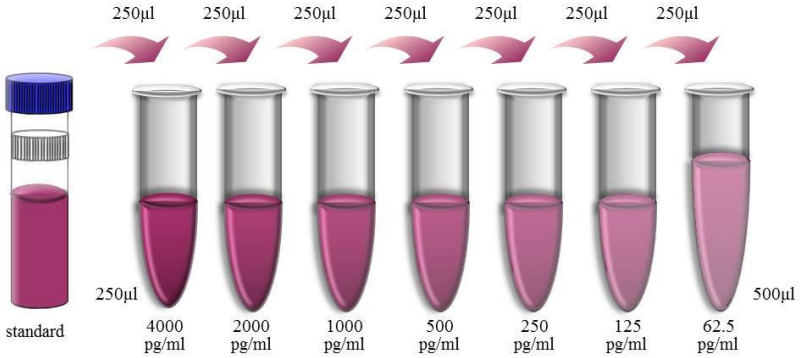
If your samples have high uPAR content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

Human uPAR Standard

Reconstitute **Human uPAR Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 8000 pg/ml).
Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.
Use polypropylene tubes.

For serum/plasma samples, mixing *concentrated human uPAR standard* (250 µl) with 250 µl of *Standard Diluent* creates the high standard (4000 pg/ml). Pipette 250 µl of *Standard Diluent* into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. *Standard Diluent* serves as the zero standard (0 pg/ml).

For cell culture supernates, mixing *concentrated human uPAR standard* (250 µl) with 250 µl of cell culture medium creates the high standard (4000 pg/ml). Pipette 250 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.
2. Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.
3. Add 300 µl *Washing Buffer (1×)* per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess *Washing Buffer (1×)*. Use the microwell strips immediately after washing. **Do not allow wells to dry.**
4. Add 100 µl of 2-fold diluted *Standard* in duplicated.
5. Add 90 µl of *Assay Buffer (1×)* and 10 µl sample to the sample well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
6. Add 50 µl of diluted *Detect Antibody* to each well.
7. Seal the plate with an *adhesive film*. Incubate at room temperature (18 to 25℃) for 2 hours on a microplate shaker set at 300 rpm.
8. Aspirate each well and wash by filling each well with 300 µl *Washing Buffer (1×)*, repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining *Washing Buffer (1×)* by aspirating or decanting. Invert the plate and tap it against clean paper towels.
9. Add 100 µl of diluted *Streptavidin-HRP* to each well.
10. Seal the plate with a fresh *adhesive film*. Incubate at room temperature (18 to 25℃) for 45 minutes on a microplate shaker set at 300 rpm.
11. Repeat aspiration/wash as in step 8.

12. Add 100 µl of *Substrate Solution* to each well. Incubate for 10 - 30 minutes at room temperature. Protect from light.
13. Add 100 µl of *Stop Solution* to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
14. Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

CALCULATION OF RESULTS

Average the duplicate optical density readings for each standards and sample, then subtract the average optical density value of the zero standard.

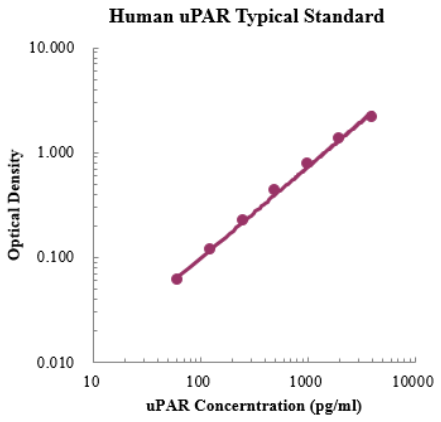
Standard Concentration as horizontal axis, optical density (OD) Value as the vertical axis, regressing the data and create a standard curve using computer software. The data may be linearized by plotting the log of the uPAR concentrations versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Note: The finally concentration of top standard is 4000 pg/ml. If instruction in this protocol have been followed samples have been diluted 1:9 (10 µl sample + 90 µl Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (×10).
If samples have been diluted following the instruction, the final dilution factor is 10. If sample have been diluted by other means, the concentration read from the standard curve must be multiplied by the appropriate dilution factor.

TYPICAL DATA

A standard curve must be run within each assay. The following standard curve is provided for demonstration only.

(pg/ml)	O.D.		Average	Corrected
0.00	0.068	0.068	0.068	
62.50	0.129	0.128	0.129	0.061
125.00	0.186	0.184	0.185	0.117
250.00	0.291	0.287	0.289	0.221
500.00	0.488	0.507	0.498	0.430
1000.00	0.852	0.840	0.846	0.778
2000.00	1.439	1.405	1.422	1.354
4000.00	2.278	2.204	2.241	2.173



SENSITIVITY

The minimum detectable dose (MDD) of uPAR is typically about 0.87 pg/ml.
The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

Intra-assay Precision (Precision within an assay)
Three serum-based and buffer-based samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three serum-based and buffer-based samples of known concentration were tested in six separate assays to assess inter-assay precision.

	Intra-assay precision				Inter-assay precision		
Sample	1	2	3		1	2	3
n	20	20	20		6	6	6
Mean (pg/ml)	242.2	735.3	2372.1		233.5	723.1	2298.9
Standard deviation	11.5	22.2	97.2		11.6	15.3	73.1
CV (%)	4.7	3.0	4.1		5.0	2.1	3.2

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human uPAR into five health human serum samples. The un-spiked serum was used as blank in these experiments.
The recovery ranged from 87 % to 114 % with an overall mean recovery of 101 %.

LINEARITY

To assess the linearity of the assay, five serum samples were spiked with high concentration of uPAR in human serum and diluted with Standard Diluent to produce samples with values within the dynamic range of the assay.

	Average (%)	Range (%)
1:2	103	96 - 112
1:4	109	101 - 115
1:8	109	101 - 111
1:16	109	99 - 114

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human uPAR produced at BioOcean.

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of uPAR in this assay. No medical histories were available for the donors used in this study.

Sample Matrix	Number of Samples Evaluated	Range (ng/ml)	Detectable (%)	Mean of Detectable (ng/ml)
Serum	30	5.7 - 15.7	100	9.3

Note: The sample range is non-physiological range. The sample range of healthy human will difference according to geographical, ethic, sample preparation, and testing personnel, equipment varies. The above information is only reference.

SPECIFICITY

This kit could assay both natural and recombinant human uPAR. A panel of substances listed below were prepared at 1 ng/ml in Standard Diluent to determine cross-reactivity. Preparations of the following substances at 1 ng/ml in a mid-range rhuPAR control to determine interference. No significant cross-reactivity or interference was observed.

Human		Mouse	Rat
ANG	IL-2	GM-CSF	IFN-γ
AR	IL-3	IL-1α	IL-1β
CNTF	IL-4	IL-1β	IL-4
EGF	IL-5	IL-3	IL-6
Epo	IL-6	IL-4	IL-10
FGF-4	IL-7	IL-5	TNF-α
FGF-5	IL-8	IL-6	
FGF-6	IL-9	IL-7	
G-CSF	IL-10	IL-9	
GM-CSF	IL-11	IL-10	
IFN-γ	IL-12	IL-13	
IL-1α	IL-13	TNF-α	
IL-1β	VEGF	uPAR	

PLATE LAYOUT